***Helicobacter pylori* Gastritis: A Histopathological and Bacteriological Study**

J.T. Anim^a V.O. Rotimi^b N. Al-Sobkie^c A. Prasad^a
A.A. Pazhoor^b B. John^a T.D. Chugh^b A.H. Gaber^c A. Mashali^c

Departments of ^aPathology and of ^bMicrobiology, Faculty of Medicine, Kuwait University,
and ^cUnit of Gastroenterology, Mubarak Al-Kabeer Hospital, Kuwait

Key Words

Helicobacter pylori · Histopathology ·
Immunohistochemistry · Bacterial culture ·
Antibiotic susceptibility · Urease test

Abstract

Objective: The primary objective of this study was to investigate five readily available methods for the diagnosis of *Helicobacter pylori* infection (both invasive and non-invasive) in order to establish the local definition of the disease in our hospital because of the regional variations in the prevalence, strains of organism and antibiotic susceptibility.

Methods: Two histological methods (HE and immunohistochemistry, IHC) and microbiological methods (culture, urease test and Gram stain of tissue smears) for processing gastric antral biopsies were compared in a prospective study in 115 consecutive pa-

tients. *H. pylori* positivity by these methods was correlated with histopathological changes in the antral biopsies. Susceptibility of the *H. pylori* isolates to various antibiotics was performed by the modified disc diffusion test. **Results:** There was no significant difference between HE and IHC in the demonstration of *H. pylori*. Using culture as the 'gold standard' for specificity, both had specificity of 91 and 86%, respectively. However, when HE was used as a standard for sensitivity, the other methods had the following sensitivities: culture 69%, Gram stain 86%, biopsy urease 85%, and IHC 91%. Higher density of *H. pylori* infection correlated with higher culture positivity and more with the presence of active chronic gastritis than with chronic inactive gastritis. Of the antibiotics tested, only metronidazole showed appreciable resistance against the *H. pylori* isolates. **Conclusion:** We recommended the use of HE and

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Prof. J.T. Anim, MD, FRCPath, Department of Pathology
Faculty of Medicine, Kuwait University, PO Box 24923
Safat 13110 (Kuwait)
Tel. +965 5319476, Fax +965 5338905
E-Mail anim@hsc.kuniv.edu.kw

culture in the definition of *H. pylori* infection at the local level, together with drug sensitivity testing to ensure an appropriate eradication strategy.

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Introduction

Helicobacter pylori infection can be diagnosed by histological examination of stained tissue sections, stained smears, cultural technique, serological evidence of immune response and biochemical assays based on its characteristic metabolic activities. The histological demonstration of *H. pylori* in gastric biopsy specimens provides high sensitivity [1, 2], especially when haematoxylin and eosin (HE) stain is supplemented with other special stains [3, 4]. Microbiological culture of *H. pylori* has 100% specificity, although its sensitivity varies widely because of problems related to transport, processing and culture of the fragile organisms [5]. In addition to histology and culture, other biopsy-related tests have been used in the investigation of *H. pylori*-related gastroduodenal disease. These include the urease test on biopsy or culture specimens and the more recently developed polymerase chain reaction methods [6, 7]. Non-endoscopy-related methods include the ^{13}C urea breath test and serology using enzyme-linked immunosorbent methods [3, 6–9]. Many workers advocate the concurrent use of at least two of the above methods (commonly histology and culture) in order to optimise the diagnostic yield and to define a gold standard for the identification of *H. pylori* infection in gastroduodenal biopsies. Specificity and sensitivity have been evaluated for these methods [1, 7, 9].

In Kuwait, we have reported a prevalence rate of 81.7% for *H. pylori* in a retrospective histopathological study of all cases presenting

to one hospital over a 1-year period [10]. This compares well with reported rates in the literature [3, 11]. However, a prospective study correlating histopathology with culture studies and endoscopic findings is necessary in the Kuwait community, not only to provide baseline data, but also to provide a basis for management policies of the disease complex in this locality. Culture of the organisms has been advocated on a regional basis because it permits testing of antibiotic sensitivity, a procedure which is assuming increasing importance on account of strain variation and the reported emergence of resistant strains [12]. Thus, important recommendations can be made for primary treatment at the local or regional level. Culture of the organisms also forms a basis for subsequent typing and subtyping of *H. pylori* strains to determine those commonly encountered in any locality [11]. In this paper we report the correlation between the histological and microbiological findings and endoscopic features in dyspeptic patients requiring upper gastrointestinal (GI) endoscopy in our hospital.

Materials and Methods

Patients presenting to the Mubarak Al-Kabeer Hospital, Kuwait, over a 3-month period with upper GI symptoms and who required upper GI endoscopy were included in the study. No attempt was made at the selection of subjects either based on clinical presentation or endoscopic findings. A total of 118 subjects were thus included in the study. In addition to basic demographic data (age, sex, ethnicity), endoscopic findings were recorded by the endoscopist at the time of endoscopy. Four biopsies were taken from the antral mucosa of each subject. Two biopsy pieces were placed in normal saline and sent immediately to the Microbiology Laboratory for processing and culture. The remaining two pieces were placed in 10% buffered formalin and sent to the Histopathology Laboratory for processing.

Microbiology Investigations

To ensure uniformity and reproducibility of procedures, all samples were handled by one senior technologist throughout the study period.

Biopsy Urease Test. A portion of the two biopsy pieces was suspended in 0.1% phenol red solution containing 10% urea and incubated at 37°C for 3 h. Thereafter, it was kept at room temperature up to 24 h and examined for colour change. When the dye solution changed to red within 24 h the specimen was adjudged to be *H. pylori*-positive.

Bacterial Culture. A portion of the biopsy pieces was ground in brain heart infusion broth (Difco) and the homogenate streaked on tryptic soy agar (Difco) supplemented with 10% (vol/vol) horse blood. Inoculated plates were incubated at 37°C in an atmosphere of 5% O₂ and 10% CO₂, in an anaerobic jar (gas pack system without catalyst) for a minimum period of 4 days. Plates not showing any growth were reincubated immediately for a further period of 6 days. The material used for the inoculum was also smeared and Gram-stained. Representative colonies that were Gram-negative curved rods, oxidase-positive, catalase-positive and urease-positive were considered as *H. pylori* [13].

Susceptibility Testing. Antibiotic susceptibility testing of the isolates was done by the modified Kirby-Bauer disc diffusion technique using Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md., USA) supplemented with 5% horse blood [14, 15]. Briefly, a sterile cotton-wool swab was dipped into a saline suspension of the organism equivalent to a 2.0 McFarland standard turbidity (containing approximately 1×10^8 CFU/ml). The swab was used to streak the surface of the agar plates in three directional strokes and allowed to dry for 2–3 min. Discs containing the following antibiotics were then placed onto the agar surface: amoxicillin/clavulanic acid (25 µg), ampicillin (10 µg), cephalothin (30 µg), ciprofloxacin (5 µg), clarithromycin (15 µg), erythromycin (15 µg), metronidazole (5 µg) and tetracycline (30 µg). The plates were incubated at 37°C for 5 days in the presence of 10% CO₂, after which the diameter of the zones of inhibition was measured. Susceptibility or resistance was interpreted according to the recommendation by the National Committee for Clinical Laboratory Standards [16]. Since there is no standard zone size established for metronidazole, the zone size recommended by DeCross et al. [14] was used (i.e. <15 mm = resistant; ≥ 15 mm = susceptible). Known in-house sensitive strains of *H. pylori* and *Escherichia coli* ATCC 25922 were used as controls.

Histopathological Methods

The formalin-fixed gastric antral tissue was processed routinely into paraffin and 4- to 5-µm sections cut for staining. One section was stained with HE. A modification of the prolonged haematoxylin method of Tazawa and Tsutsumi [17] was applied. Thus, haematoxylin staining was extended from the usual 5 min to 12 min. Another section was coated with APES for immunohistochemical staining for *H. pylori*, using purified polyclonal antiserum (Dako B0471, Glostrup, Denmark). After blocking endogenous peroxidase with 3% H₂O₂ in methanol, the primary antiserum was followed by peroxidase-antiperoxidase reaction as prescribed by the manufacturer. Colour development was by diaminobenzidine.

Histopathological changes were assessed, looking for inflammatory responses to the presence of *H. pylori*, namely: chronic gastritis with or without activity, lymphoid hyperplasia and the presence of intestinal metaplasia. These changes have been defined earlier [10]. *H. pylori* colonisation was graded per high power field ($\times 40$ objective) for both HE and immunohistochemistry (IHC) into: grade 0 = no organism; grade 1 = <5 organisms; grade 2 = 5–10 organisms; grade 3 = >10 organisms. The organisms were counted from the most heavily colonised crypt. Grading of *H. pylori* colonisation was done by 2 pathologists and a consensus was arrived at when there was any difference. In general, there was good agreement in the grading by the 2 pathologists. Histological examination was done blind, without prior knowledge of the culture results.

Statistical Methods

Simple descriptive statistical methods such as chi-square test were used to compare parameters. SPSS version 8.0 was used and Z test for proportion applied to test the significance of associations; p values of 0.05 and below were considered as significant.

Results

A total of 118 patients were studied. Their ages ranged between 15 and 72 years with a mean of 39 years. There were 76 males and 42 females (ratio 1.8:1). Satisfactory culture results were available for 115 of the 118 cases. In biopsies from these 115 patients, *H. pylori* was demonstrated in 85 (73.9%) using HE and in 77 (67%) using IHC. There was no sig-

nificant difference between the two histological methods for the detection of *H. pylori* colonisation of the gastric antrum in our patients. Culture was positive in 59 of the 115 cases (51.3%). Table 1 shows the correlation between *H. pylori* density in the antral biopsies as defined by HE and IHC and culture positivity. In nearly all cases, except grade 0, there was a positive correlation between the *H. pylori* density and culture positivity.

Table 2 shows correlation between the detection of *H. pylori* using HE and histopatho-

logical lesions in the gastric mucosa. Higher prevalence of *H. pylori* colonisation (94%) was demonstrated in 50 biopsies with active chronic gastritis with or without the presence of lymphoid hyperplasia, compared with 76% in inactive chronic gastritis in 47 patients ($p = 0.02$). Correlation between histopathological lesion and the different tests used to detect *H. pylori* are also summarised in table 2. Higher positive culture rates were also observed in active chronic gastritis (72%), when compared with chronic gastritis without activity (45%; $p < 0.02$).

H. pylori was demonstrated in 73 (63.4%) and 72 (62.6%) biopsies, respectively, by the smear and biopsy urease tests (table 2). Using HE as standard for sensitivity, both urease and smear demonstration of *H. pylori* showed higher sensitivity (84.7 and 85.9%, respectively) compared to culture (69.4%). As with the culture, *H. pylori* was demonstrated more frequently in active chronic gastritis compared with chronic gastritis without activity, using the urease test ($p < 0.03$) and Gram stain of smears ($p < 0.05$). The diagnostic relationship between the results of culture, Gram stain, HE and IHC is demonstrated in table 3.

Table 1. Correlation between *H. pylori* density by HE and immunohistochemistry vs. positive culture results in 59 cases

<i>H. pylori</i> grade	Number (%) positive by stain/number positive by culture	
	HE	IHC
0	11/15 (73.3)	11/19 (58)
1	4/5 (80)	1/1 (100)
2	6/6 (100)	3/3 (100)
3	33/33 (100)	36/36 (100)
Total	54/59 (91.5)	51/59 (86.4)

Table 2. Correlation between histopathological changes in gastric antral biopsies and histological and bacteriological methods for detection of *H. pylori*

Histological lesion (n)	Number (%) positive for <i>H. pylori</i>			
	Gram stain	biopsy urease	culture	HE
Active chronic antral gastritis (20)	16 (80)	15 (75)	14 (70)	17 (85)
Active chronic antral gastritis with lymphoid hyperplasia (30)	25 (83)	26 (87)	22 (73)	30 (100)
Chronic inactive antral gastritis (12)	8 (67)	8 (67)	7 (58)	9 (75)
Chronic inactive antral gastritis with lymphoid hyperplasia (35)	22 (63)	21 (60)	14 (40)	27 (77)
Intestinal metaplasia (3)	1 (33)	1 (33)	1 (33)	1 (33)
Normal (15)	1 (6.7)	1 (6.7)	1 (6.7)	1 (6.7)
Total (115)	73 (63.4)	72 (62.6)	59 (51.3)	85 (73.9)

A 91% correlation was found between culture results and microscopy in 55 patients, using Gram stain and HE stain. There was an 86% agreement among culture, Gram stain and IHC. The specificity of HE or IHC was 91.5 and 86.4%, respectively.

Table 4 summarises the susceptibility of the *H. pylori* isolated in this study. With the exception of metronidazole, to which 17.3% of the cases tested showed a resistance, *H.*

pylori appeared to be susceptible to all the other antibiotics in common use for *H. pylori* eradication at the present time, e.g. ampicillin, amoxycillin, clavulanic acid and clarithromycin.

Discussion

Our results show that in the hands of an experienced histopathologist, the difference between the two histological methods (HE and IHC) for demonstrating the presence of *H. pylori* in gastric antral biopsies is not significant. The prolonged haematoxylin staining has been recommended by Tazawa and Tsutsumi [17] to improve staining of the organisms and facilitate their recognition even in sparsely colonised tissues.

Moreover, HE is known to provide excellent accuracy when more than minimal *H. pylori* density is present [18]. In this study, HE showed the highest sensitivity for demonstration of the organisms, although some workers claim that IHC may be of greater value where *H. pylori* density is low, as is often the case after eradication treatment [19]. One other advantage of HE is that histopathological changes in the gastric mucosa that accompany *H. pylori* infection are best demonstrated with this stain [1, 6]. Others have recommended the addition of other methods to HE staining to demonstrate *H. pylori*. McNulty and Watson [20] reported in 47 specimens a 92% agreement between Gram stain, culture and histological examination, using HE. These results correlate very well with ours. Thus, we are inclined to agree with those authors who consider the combination of culture and histological tests as the 'gold standard' for *H. pylori* infection [5, 21]. It is, however, accepted that, of the many methods (both direct and indirect) available for the diagnosis of *H. pylori*, a combination of at

Table 3. Relationship between culture, Gram stain and histological demonstration of *H. pylori* in gastric antral biopsies of 55 patients

Detection technique	Biopsies	
	n	%
Culture+	55	100
Culture+ Gram stain+ HE+	50	91
Culture+ Gram stain+ HE-	2	4
Culture+ Gram stain- HE+	3	6
Culture+ Gram stain- HE-	0	0
Culture+ Gram stain+ IHC+	47	86
Culture+ Gram stain+ IHC-	5	9
Culture+ Gram stain- IHC+	3	6
Culture+ Gram stain- IHC-	0	0

Table 4. Susceptibility of *H. pylori* to antimicrobial agents

Antimicrobial agents	Isolates tested	
	n	%
Metronidazole	52	82.7
Tetracycline	52	98.1
Ampicillin	42	100
Cephalothin	54	98.1
Clarithromycin	40	100
Ciprofloxacin	50	100
Erythromycin	52	98.1
Amoxicillin/clavulanic acid	16	100

least two different techniques should be used in order to optimise the diagnostic yield [22].

The data generated in this study show that the majority of patients had high (grade 3) density of *H. pylori* in the gastric antral biopsies. Also, there is a higher frequency of isolation as well as high density of *H. pylori* in active chronic antral gastritis when compared to chronic inactive antral gastritis. This is more striking when active chronic gastritis without lymphoid hyperplasia is combined with active gastritis with lymphoid hyperplasia and compared with the inactive counterpart ($p < 0.03$). The presence of activity in the gastric mucosa is thus an important factor in *H. pylori* colonisation and supports the role of this organism in the pathogenesis of gastritis [3].

H. pylori was demonstrated by culture in 59 (51.3%) of the cases. As expected, higher culture rates were seen in those with high density of *H. pylori* colonisation and positive cultures also showed positive correlation with active chronic gastritis. Thus, a higher proportion of cases of gastritis with activity yielded positive cultures compared with those without activity ($p < 0.02$). This further supports the pathogenetic relationship between *H. pylori* colonisation and active inflammatory lesions of the gastric mucosa. Both urease and smear tests also showed similar correlation.

In this study, the modified Kirby-Bauer disc method of testing for antibiotic susceptibility was adopted because it is easy to perform, has been shown to correlate well with minimum inhibitory concentration obtained by the agar dilution method, and has been demonstrated to be clinically applicable [14, 15]. With the exception of metronidazole to which as many as 17.3% of our isolates demonstrated resistance, the local *H. pylori* strains isolated from our patient population with dyspepsia in Kuwait appear to be susceptible to all the antibiotics in common usage world-

wide for the eradication of the organism. Resistance to metronidazole is an increasing and constitutional problem in the treatment of *H. pylori* infection. Reports of resistance to this drug range from 11 to 70% with even higher rates being reported from some developing countries [3]. Even though the resistance rate of 17.3% reported in this study is in the lower end of the range, it is still unacceptable, particularly when the drug is being considered for empirical therapy. However, with the availability of many alternatives, choice of antibiotics should not pose any serious problems in Kuwait at the present time, although reports of resistance to other antibiotics are present in the literature. Our study serves to underscore the need to study the culture and drug susceptibility characteristics of the organism at the local and regional level [7, 22, 24]. This need is buttressed by the fact that *H. pylori* eradication has become a universally accepted form of therapy, not only for gastritis and peptic ulcer, but also because *H. pylori* eradication is now known to reduce the risk of developing gastric lymphoma and gastric carcinoma, both of which have been associated with *H. pylori* infection.

In conclusion, as a result of the evidence-based data generated from this study, we recommend the use of HE and culture in the definition of *H. pylori* infection at the local level, together with antimicrobial susceptibility testing to ensure an appropriate eradication strategy.

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